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Effect of Ammonium on Nitrate Utilization by Roots of Dwarf Bean

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ABSTRACT

The effect of exogenous NH_4^+ on NO_3^- uptake and *in vivo* NO_3^- reductase activity (NRA) in roots of *Phaseolus vulgaris* L. cv Witte Krombek was studied before, during, and after the apparent induction of root NRA and NO_3^- uptake. Pretreatment with NH_4Cl (0.15–50 millimolar) affected neither the time pattern nor the steady state rate of NO_3^- uptake.

When NH_4^+ was given at the start of NO_3^- nutrition, the time pattern of NO_3^- uptake was the same as in plants receiving no NH_4^+. After 6 hours, however, the NO_3^- uptake rate (NUR) and root NRA were inhibited by NH_4^+ to a maximum of 45% and 60%, respectively.

The response of the NUR of NO_3^--induced plants depended on the NH_4Cl concentration. Below 1 millimolar NH_4^+, the NUR declined immediately and some restoration occurred in the second hour. In the third hour, the NUR became constant. In contrast, NH_4^+ at 2 millimolar and above caused a rapid and transient stimulation of NO_3^- uptake, followed again by a decrease in the first, a recovery in the second, and a steady state in the third hour. Maximal inhibition of steady state NUR was 50%. With NO_3^--induced plants, root NRA responded less and more slowly to NH_4^+ than did NUR.

Methionine sulfoximine and azaserine, inhibitors of glutamine synthetase and glutamate synthase, respectively, relieved the NH_4^+ inhibition of the NUR of NO_3^--induced plants. We conclude that repression of the NUR by NH_4^+ depends on NH_4^+ assimilation. The repression by NH_4^+ was least at the lowest and highest NH_4^+ levels tested (0.04 and 25 millimolar).

NO_3^- is the main source of N for crop plants, including legumes (23). At present there is much concern about the ecological and nutritional impact of NO_3^- fertilizers. A better understanding of the physiological processes by which the plant responds to the presence of NO_3^- in its environment may permit a more intelligent use of NO_3^-. Despite its biological and agricultural significance, detailed studies on the process of NO_3^- acquisition by plants are fairly recent and insight into the regulation of NO_3^- uptake is still in its infancy (12).

The process of NO_3^- uptake by higher plants differs from that of other inorganic nutrients. For example, the fate of NO_3^- in the plant may be accumulation as well as conversion and both uptake and reduction have characteristics of induction. Against this background, we started an investigation into the initial events of NO_3^- utilization in dwarf bean, a species which is very flexible in the choice of its N source. Our previous work has identified NO_3^- uptake and reduction of NO_3^- to NO_2^- in the root system as two key steps in NO_3^- utilization by N-depleted dwarf bean (3, 6).

Utilization of NO_3^- encompasses all processes that deliver exogenous NO_3^--N to its final destination which may range from deposition in the root to biosynthesis in the shoot. Assimilation of NO_3^- has a number of obligatory chemical intermediates such as NO_2^-, NH_4^+, amides, and amino acids. Each intermediate may affect the utilization of NO_3^- and thus serve as a metabolic regulator (6).

Numerous reports deal with the effect of NH_4^+ on NO_3^- uptake in higher plants. The effect varies from no effect in detached barley roots (25) to a partial inhibition in maize (13), tomato (14), and sweet potato (16). Recent findings indicate that NH_4^+ may affect the influx (13) as well as the efflux component (9) of net NO_3^- uptake.

It has been proposed that the repression of NO_3^- uptake by NH_4^+ in lower plants is not caused by NH_4^+ as such. In algae and bacteria, the action of NH_4^+ on NO_3^- uptake seems to be regulated either by an assimilatory enzyme (GS[2], 18) or a product of NH_4^+ assimilation (27), *e.g.* glutamine (1). For maize, Rufty *et al.* (22) suggested that inhibitory factors generated during NH_4^+ assimilation may inhibit NO_3^- uptake.

The effect of NH_4^+ on NRA during and after induction has been reported to be none, positive, or negative, depending on species, genotype, tissue, and nutrient conditions (26). It is unclear whether the effect on root NO_3^- reduction is related to the effect of NH_4^+ on uptake.

The aim of the present work was to elucidate the role of NH_4^+ in the initial utilization of NO_3^- by roots of dwarf bean. Short reports of our results have appeared (4, 8).

MATERIALS AND METHODS

Plant Cultivation and Experiments. *Phaseolus vulgaris* L. cv Witte Krombek was germinated in perlite and grown unnodulated for 7 ± 1 d on an N-free basal medium (BM) at pH 5.0 ± 0.2 under a 16-h photoperiod (30 w m^{-2}) at 65% RH and 20°C. NO_3^- nutrition started by the addition of $Ca(NO_3)_2$, with or without NH_4Cl, to plants grown in BM. During uptake experiments, the concentration of NO_3^- was 0.15 or 0.4 mM which rendered the NUR independent of NO_3^- concentration (7). Plant to solution ratios were chosen such that the ambient pH fluctuations were kept within the range 4.9 to 5.4. For further details on plants and experiments, we refer to earlier papers (3, 5–7). All experiments were repeated at least twice and results are expressed per unit of root dry mass.

Analytical Procedures. Uptake of NO_3^- and NH_4^+ was meas-

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[2] Abbreviations: GS(A), glutamine synthetase (activity); BM, basal medium; NUR, NO_3^- uptake rate; GDH(A), glutamate dehydrogenase (activity); LN_2, liquid nitrogen; NR(A), NO_3^- reductase (activity); 2-OG, 2-oxoglutarate; MSO, methionine sulfoximine; AS, azaserine; GOGAT, glutamine-oxoglutarate aminotransferase (glutamate synthase).

ured by ambient depletion. NO_3^- was determined continuously or discontinuously by UV spectrophotometry, or colorimetrically with Szechrome reagent or reduction with hydrazine as indicated previously (6, 7). Data obtained by UV analyses were checked with data from other assays and only used when the difference in concentration obtained by independent methods was less than 6%. The Berthelot reaction was used to assay ambient NH_4^+ (2), and plant NH_4^+ was measured in a cold water extract of LN_2-powdered tissue made with an Ultraturrax pistol blender. The extracts were steam-distilled at pH 10.5 into boric acid and the trapped NH_3 was determined acidimetrically (2). Under these conditions, amide breakdown was <5% and NH_4^+ recovery was >98%.

NO_3^- reductase activity was measured by an anaerobic *in vivo* test (5) that gave results in agreement with the rate of *in situ* reduction (3). The activity of GS and GDH was measured *in vitro* in an extract prepared with a mortar and pestle from fresh LN_2-powdered roots mixed with sand. The extraction buffer (pH 7.2, 4°C) contained imidazole, DTT and Na_2EDTA at concentrations of 50, 1, and 0.5 mM, respectively. Crude extracts were centrifuged (2°C, 5 min, 5000*g*) and kept on ice until the enzyme assays. The synthetase reaction of GS was measured by the formation of L-glutamate-5-monohydroxamate after 30 min of incubation at 30°C in a pH 7.2 buffer containing K-glutamate, imidazole, $MgSO_4$, hydroxylamine, and ATP at concentrations of 80, 50, 20, 20, and 8 mM, respectively. The reaction was stopped by addition of a mixture of TCA and $FeCl_3$ in HCl (20). After centrifugation (5 min, 5000*g*), the absorbance was measured at 546 nm. The absorbance of extracts incubated without ATP was subtracted and the absorbance difference compared with standard series of L-glutamate-5-monohydroxamate subjected to the same procedure as the plant extracts.

The NADH-dependent activity of GDH was estimated spectrophotometrically at 340 nm by measuring the oxidation of NADH by a crude enzyme extract mixed with a pH 7.2 buffer which contained Tricine, $(NH_4)_2SO_4$, 2-OG, $CaCl_2$, and NADH in final concentrations of 50, 50, 10, 1, and 0.2 mM, respectively. NADH consumption by other enzyme systems was corrected for by measuring GDHA against a reference cuvette with identical composition, except that K_2SO_4 replaced $(NH_4)_2SO_4$ and 2-OG was omitted. The activity in the sample cuvette was 5 to 6 times higher than the activity in the reference cuvette.

RESULTS

Pretreatment with NH_4^+. An 18-h pretreatment with NH_4Cl (0.15–50 mM) did not affect the duration of the apparent induction phase of NO_3^- uptake (data not shown). Induction took about 6 h in all treatments, and the shape of the NUR-time curve (*cf.* Fig. 1) was not significantly altered by the NH_4^+ history. After induction of NO_3^- uptake, the NUR did not differ significantly between NH_4^+-pretreated and control plants (data not shown).

As compared with control plants, initial potential NRA (5) was unaffected in primary leaves, but 50% lower in roots of NH_4^+-pretreated (18 h, 0.15 and 10 mM) plants. Two and 6 h after NO_3^- supply, however, actual NRA equaled potential NRA in roots and leaves, and no pretreatment effect of NH_4^+ was observed (data not shown).

Concomitant Supply of NH_4^+ and NO_3^-. When N nutrition was initiated with 0.15 mM NH_4NO_3, the time pattern of NO_3^- uptake was the same as with $Ca(NO_3)_2$ (Fig. 1), but steady state NUR was about 30% lower with NH_4NO_3. The inhibition was aggravated at higher exogenous NH_4^+ levels (Fig. 2) and became maximal (40–50%) between 1 and 10 mM. NH_4^+ levels between 1 and 10 mM had no effect on the time pattern of NO_3^- uptake during concomitant supply of the two N sources (data not shown). NH_4^+ uptake was not affected by NO_3^- (Fig. 1). In the absence of each other, NH_4^+ uptake exceeded NO_3^- uptake by about 10% after 6 h. On NH_4NO_3, the rate of NH_4^+ uptake exceeded the NUR by about 50%.

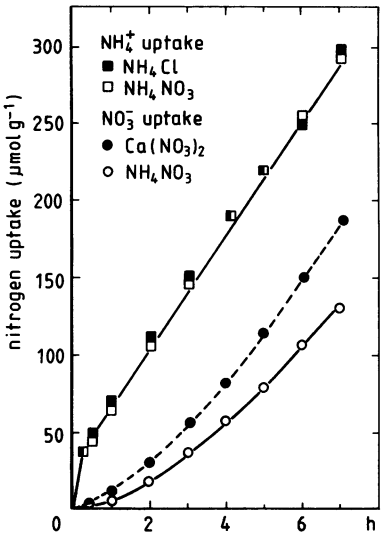

FIG. 1. Uptake of NO_3^- and NH_4^+ by dwarf bean in the absence and presence of each other. N sources added at $t = 0$, 0.15 mM. The average coefficients of variation for the data are 9% (NO_3^-) and 4% (NH_4^+).

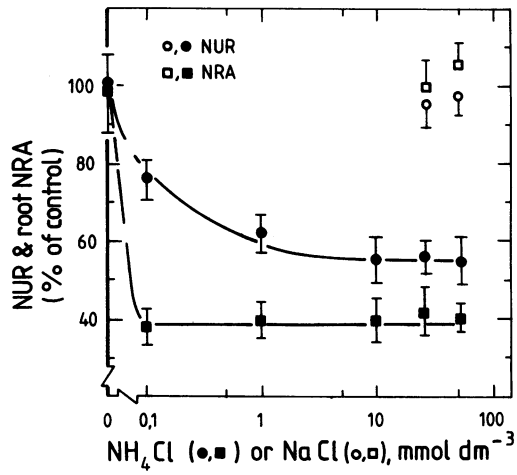

FIG. 2. Effect of NH_4^+ concentration on NO_3^- uptake rate (NUR) and NO_3^- reductase activity (NRA) of dwarf bean roots after 6 h of NO_3^- (0.15 mM) supply in the absence or presence of NH_4Cl. Data are given ± SD, $n = 3$ (NUR) or $n = 5$ (NRA). Control values: NUR, 34.8 µmol NO_3^- g^{-1} h^{-1}; NRA, 11.2 µmol NO_2^- g^{-1} h^{-1}. Values of NaCl controls are given for 25 and 50 mM. Controls with KCl at 2 and 10 mM yielded similar values.

Root NRA was curtailed with about 60% by NH_4^+, irrespective of the exogenous NH_4Cl concentration (0.1–50 mM; Fig. 2).

NH_4^+ Supply to NO_3^--Induced Plants. Plants grown with NO_3^- for 18 h had a constant NUR and NRA in leaves and roots (5) and were used to study the postinduction effect of NH_4^+ on uptake and reduction of NO_3^-.

Addition of NH_4^+ affected the NUR immediately, and the NH_4^+ effect varied with the level of applied NH_4^+ (Fig. 3). At concentrations below 1 mM, the NUR declined immediately but some restoration occurred in the 2nd h. In the 3rd h, the rate became constant. At concentrations above 1 mM, there was an initial and transient stimulation of NO_3^- uptake, but after 15 to 30 min the same time pattern as with lower NH_4^+ levels prevailed. Both the initial stimulation and the eventual steady state

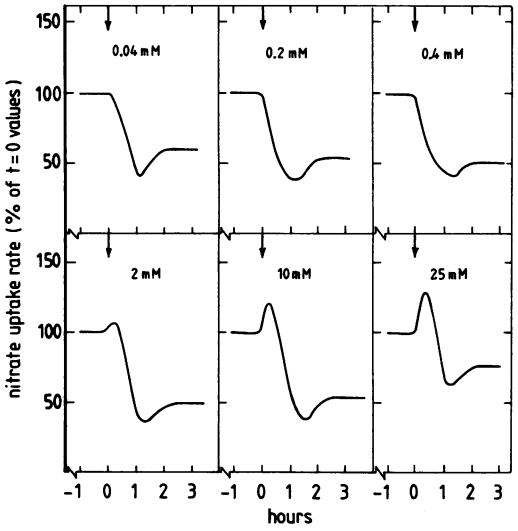

FIG. 3. Effect of NH_4^+ on the NO_3^- uptake rate (NUR) of dwarf bean. NO_3^--induced plants (18 h, 0.4 mM) were supplied with NH_4Cl (concentration indicated in figure) at $t = 0$ (arrows). The NUR was computed from continuous recordings of the disappearance of NO_3^- *versus* time and expressed as per cent of the initial rate ($t = 0$) for each NH_4^+ concentration. Control values varied between experiments from 37 to 48 μmol NO_3^- g^{-1} h^{-1}.

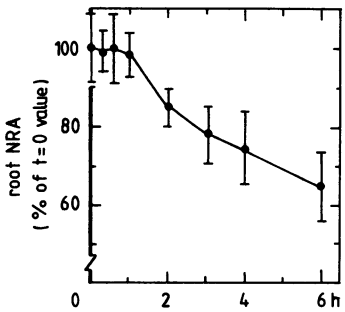

FIG. 4. Effect of NH_4^+ on NO_3^- reductase activity (NRA) of roots of NO_3^--induced (18 h, 2 mM) dwarf bean. NH_4Cl (2 mM) was added to the NO_3^- medium at $t = 0$. Data are given as per cent of the control ($t = 0$; 20.8 μmol NO_2^- g^{-1} h^{-1}) ± SD ($n = 5$).

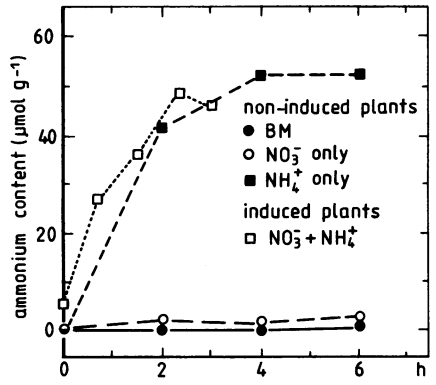

FIG. 5. NH_4^+ content of dwarf bean roots. N was given as NH_4^+ or NO_3^- (0.4 mM at $t = 0$) and control kept on basal medium (BM), or as NH_4^+ (2 mM) given at $t = 0$ together with NO_3^- (0.4 mM) to NO_3^--induced (18 h, 2 mM) plants. The average coefficient of variation for the data is 12%.

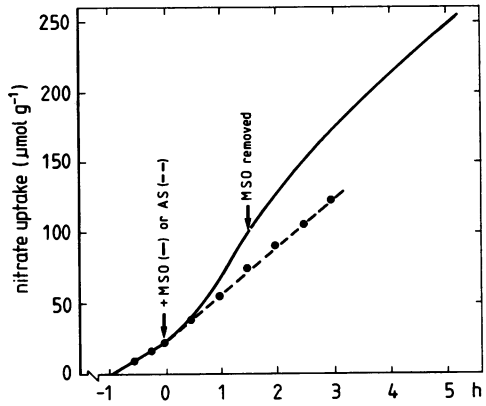

FIG. 6. Effect of methionine sulfoximine (MSO) and azaserine (AS) on NO_3^- uptake by dwarf bean. Nitrate-induced plants (18 h, 0.4 mM) were supplied with NH_4Cl (2 mM) at $t = -3$ h and, at $t = 0$, MSO or AS were given (pH 5.0, 1 mM). MSO was removed after 1.5 h (2 × 1 min wash with MSO-free medium). NH_4^+ and NO_3^- were present throughout and NO_3^- uptake in the MSO experiment was computed from a continuous recording of the NO_3^- concentration *versus* time.

rate were consistently highest at 25 mM NH_4^+. At 0.04 mM NH_4^+, the NUR after 3 h was also consistently higher than at 2 mM NH_4^+. Maximum inhibition of the NUR over the whole NH_4^+ concentration range tested was about 50%. Parallel experiments with NaCl or KCl proved that the effect of NH_4Cl was due to the NH_4^+ ion (data not shown). Removal of exogenous NH_4^+ (2 mM) after 3 h resulted in a further decrease of the NUR. Uptake recovered, however, within 3 h to the NH_4^+-inhibited rate (data not shown). Washing of roots during NO_3^- uptake in the absence of NH_4^+ did not alter the NUR. Similarly, a second wash, 3 h after NH_4^+ removal, did not affect the NUR. Prolonged NH_4^+ supply (3–10 h at 0.04–25 mM) did not reduce the NUR by more than 50% (data not shown).

Root NRA, in contrast to NUR, was unaffected by 5 min of NH_4^+ supply (2 mM, data not shown). Similarly, when NH_4^+ (5 mM) was added to the incubation buffer in an *in vivo* NRA assay of NO_3^--induced roots, the rate of NO_2^- release was not influenced for at least the subsequent 45 min. At 2 mM of nutrient NH_4^+, root NRA was only significantly affected after 2 h, and the inhibition was about 35% after 6 h of mixed NH_4^+ + NO_3^- nutrition (Fig. 4).

NH_4^+ Content in Roots. Plants kept on BM contained very little NH_4^+, typically 1 μmol/g dry root, corresponding to a mean cellular concentration of about 0.1 mM. After 6 h of N supply (0.4 mM) NH_4^+ in roots increased 3-fold and 50-fold with NO_3^- and NH_4^+, respectively (Fig. 5). Accumulation of NH_4^+ was similar in roots of induced and uninduced plants for at least 3 h.

Effect of Methionine Sulfoximine and Azaserine. MSO and AS, inhibitors of GS and GOGAT, respectively, were used to assess whether the effect of exogenous NH_4^+ is exerted by NH_4^+ per se or by an event or compound related to its primary assimilation in the root. When the NUR of induced plants was about 50% inhibited by 2 mM NH_4^+ (*cf.* Fig. 3), the addition of MSO rapidly accelerated NO_3^- uptake (Fig. 6). The NUR gradually declined upon removal of the inhibitor, but the GSA was irreversibly inhibited by MSO (Fig. 7). Without removal of MSO, the NUR did not decrease for the subsequent 1 to 2 h (data not shown). When added to NH_4^+-free media, MSO did not alter the NUR of induced plants for at least 0.5 h, but caused a 30% lower NUR than in control plants after 3 h (data not shown). In mixed NO_3^- + NH_4^+ nutrition, glutamate, of which MSO is an analogue, did not stimulate the NUR (data not shown). AS also rapidly stimulated the NUR of NH_4^+-affected plants (Fig. 6).

Pretreatments (1 h, 1 mM) with MSO and AS caused a transiently decreasing initial NO_3^- uptake. After 6 h of NO_3^- supply, the NUR was 45% (MSO) and 25% (AS) of that of nonpretreated

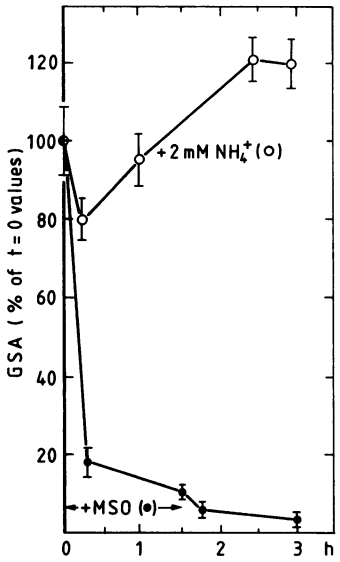

FIG. 7. Glutamine synthetase activity (GSA) in roots of NO_3^--induced (18 h, 0.4 mM) dwarf bean. Lower graph: plants were given NH_4^+ (2 mM) at $t = -3$ h, and MSO between 0 and 1.5 h, as outlined in the legend of Figure 6. Upper graph: plants were given NH_4^+ (2 mM) at $t = 0$. Data expressed as per cent of the control values ± SD ($n = 2$), which were 0.48 (lower graph) and 0.39 (upper graph) mmol glutamine g^{-1} h^{-1}.

Table I. *Effect of MSO and NH_4^+ on the NRA of Dwarf Bean Roots*

NRA of NO_3^--induced plants (18 h, 2 mM) was measured after 6 h of treatment with MSO (1 mM), NH_4^+ (2 mM), or both.

Treatment	NRA
	%
NO_3^-	100 ± 11[a]
NO_3^- + NH_4^+	71 ± 8
NO_3^- + MSO	54 ± 6
NO_3^- + MSO + NH_4^+	56 ± 5

[a] Per cent of control (NO_3^- only; 6.3 μmol NO_2^- g^{-1} h^{-1}) and given ± SD ($n = 5$).

plants, which showed an induction pattern similar to that in Figure 1 (data not shown).

Six h after the start of NO_3^- supply in the presence of MSO, root NRA was only 10% of the control. A short revival of root NRA occurred consistently after 4 h (data not shown). Root NRA of induced plants was decreased more by MSO than by NH_4^+ (Table I; *cf.* Fig. 4), and the effects of MSO and NH_4^+ did not appear to be additive.

Glutamine Synthetase and Glutamate Dehydrogenase. GSA was measured at various times during NH_4^+ inhibition of the NUR of induced plants (Fig. 7). The initial drop in GSA coincided with a transient stimulation of NO_3^- uptake by NH_4^+ and the plateau of GSA in the 3rd h coincided with a constant (about 50% inhibited) NUR and with a constant level of endogenous NH_4^+ (Fig. 5). The *in vitro* activities of GS and GDH in induced roots were measured after 3 h of NH_4^+ (0–25 mM) supply to estimate the partition of NH_4^+ assimilation in roots over the GS-GOGAT route and the GDH route (Fig. 8). At low ambient NH_4^+, the activity of GS was about 2 times higher than that of GDH. At 25 mM NH_4^+, GDHA and GSA were equal.

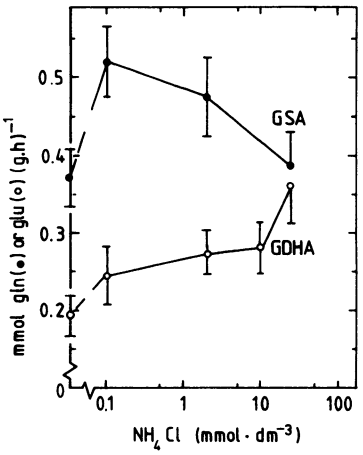

FIG. 8. Activities of glutamine synthetase (GSA) and glutamate dehydrogenase (GDHA) in roots of NO_3^--induced (18 h, 0.4 mM) dwarf bean. Enzyme activities were measured after 3 h of NH_4^+ supply at various concentrations, in the presence of NO_3^- (0.4 mM) and are given ±SD ($n = 2$).

DISCUSSION

Uptake and Accumulation of NH_4^+. Uptake of NH_4^+ proceeded at a constant rate after 0.5 h and was unaffected by an equimolar concentration of NO_3^- (Fig. 1). The initial phase probably reflects the filling of a free space (2). NH_4^+ was taken up faster than NO_3^- in the absence and in the presence of NO_3^-. After 6 h of NO_3^- supply, the concentration of NH_4^+ in the roots of about 3 μmol g^{-1} (Fig. 5) was much higher than that of NO_2^- (<0.5 μmol g^{-1} [6]) and much lower than that of NO_3^- (about 40 μmol g^{-1} [3]) under similar conditions. In induced and uninduced plants, NH_4^+ in roots approximated a plateau level of about 50 μmol g^{-1} after a few hours of NH_4^+ supply (Fig. 5).

NO_3^- Uptake. The apparent induction of NO_3^- uptake proceeded identically in the absence and presence of NH_4^+ (Fig. 1), but the NUR was rapidly and severely affected by NH_4^+ (Figs. 2 and 3). The lack of a reverse effect (Fig. 1) shows that NH_4^+ and NO_3^- are not competing for a common uptake site. Inhibition of NH_4^+ assimilation by blocking the GS-GOGAT cycle prevented the normal induction of NO_3^- uptake. The increase of the NUR from an initial low to a high constant value thus seems to require normal NH_4^+ assimilation or its products, *e.g.* newly made amino acids and proteins. An altered N status due to NH_4^+ pretreatment did not affect the induction of NO_3^- uptake. For wheat (28) and cultured tobacco cells (11), the timing of NO_3^- uptake is also independent of exogenous NH_4^+.

NH_4^+ could not repress the NUR after 6 h NO_3^- supply by more than 40% to 50% (Fig. 2). Similarly, the steady state NUR of NH_4^+-affected induced plants was at least 50% of the uninhibited rate (Fig. 3). These findings suggest that only part of the NO_3^- uptake system can be influenced by NH_4^+, and that NH_4^+ in the millimolar range causes maximal inhibition. In induced plants, NH_4^+ at 2 mM and above caused a rapid but transient increase of the NUR, and the inhibition was lower at 25 than at 0.2 to 10 mM. Rao and Rains (19) found a similar concentration-dependent effect of NH_4^+ on the NUR of barley plants. At 0.1 mM, they reported an inhibition of the NUR of about 35% whereas the NUR was higher below and above that concentration. It was recently reported for tomato that inhibition of the NUR decreases when the NH_4^+ level exceeds 50 μM (14).

NH_4^+ assimilation in higher plants usually involves the high affinity GS-GOGAT route, the low affinity GDH route only being significant at high endogenous NH_4^+ concentrations (21). Our results from *in vitro* enzyme activities in roots, and data from duck weed (21) and maize leaves (24), are consistent with the prevalence of the GS-GOGAT route at low and an increasing contribution of the GDH route at high NH_4^+ concentrations (Fig. 8). Inhibition of NO_3^- uptake may be related to the assimilation route of the absorbed NH_4^+. Apparently, a high GDHA or a low GSA is associated with less inhibition of NO_3^- uptake

by NH_4^+. Recently, labeling experiments with $^{15}NH_4^+$ have produced results that are inconsistent with any significant role of the GDH route (10, 15). Although ^{15}N data are by far more conclusive than data from *in vitro* enzyme activities, it should be noted that no ^{15}N labeling data are available for the highest NH_4^+ levels used in our experiments.

The time pattern of NH_4^+ inhibition of NUR in induced plants is consistent with an inhibitory role of the GS-GOGAT route (Fig. 7). It is also possible that an unknown critical factor, *e.g.* an amino acid, is responsible for the time pattern and concentration dependence of the inhibition by NH_4^+, but this explanation would require a lower concentration of this factor at high NH_4^+ levels.

Other evidence for the involvement of the GS-GOGAT route in the inhibition of NO_3^- uptake comes from experiments with the specific enzyme inhibitors MSO and AS (Fig. 6). MSO rapidly and irreversibly inactivated GS (Fig. 7) and caused a swift increase of the NUR of NH_4^+-inhibited plants. AS, although to a lesser extent, also relieved the NH_4^+ inhibition of NO_3^- uptake (Fig. 6). Removal of MSO gradually decreased the NUR, despite the further decrease in GSA. This effect of MSO may be due to its function as glutamate analog, since Breteler and Arnozis (in preparation) found that inhibition of NUR by glutamate was aggravated rather than relieved upon removal of the inhibiting compound. Moreover, removal of NO_2^- (6) and NH_4^+ also drastically lowered NO_3^- uptake. Continuation of an NH_4^+ effect for several hours in the absence of exogenous NH_4^+ is another indication of the involvement of NH_4^+ assimilation in the suppression of the NUR. Since MSO and AS have at least qualitatively the same effect on NO_3^- uptake, it is unlikely that the accumulation of NH_4^+, glutamine, or glutamate is responsible for the repression of the NUR. Aspects of the GS-GOGAT route other than the primary intermediates seem to be involved. A strong candidate is the ATP-requiring reaction catalyzed by GS, which may divert ATP from sustaining active NO_3^- uptake (3) to NH_4^+ assimilation, as suggested by Ohmori *et al.* for an alga (17).

NO_3^- Reductase. A lower amount of entering substrate for NO_3^- reduction was not the sole effect of NH_4^+ on root NRA. During initial NO_3^- supply, root NRA was more affected by NH_4^+ than was the NUR, and maximal inhibition of NRA was already exerted by 0.1 mM NH_4^+, the lowest concentration tested (Fig. 2). In induced plants, NUR was more affected by NH_4^+ than was NRA. NUR was affected immediately (Fig. 3), whereas root NRA started to decrease between 1 and 2 h, and to a lesser extent than the NUR, under similar conditions (Fig. 4). NUR and root NRA thus seem to be independently regulated both during and after the apparent induction of uptake and reduction of NO_3^-.

Independent of the presence of NH_4^+, root NRA of induced plants was lowered by MSO (Table I). Events or compounds associated with assimilation of NH_4^+ via the GS-GOGAT route thus were required for both induction and high activity of root NRA.

NO_3^- Uptake and N Assimilation. Uptake of NO_3^- appeared more related to NH_4^+ assimilation than to NO_3^- assimilation (Figs. 3, 4, and 6). The NO_3^- history of the plants determined whether NH_4^+ affects NUR more or less than NRA, and also determined the timing and magnitude of the response of NUR to NH_4^+ (Figs. 1–3). We therefore conclude that NH_4^+ does not exclusively affect NO_3^- utilization by the generation of a common regulator of uptake and reduction. Rao and Rains (19) similarly reached the conclusion that in barley the effect of NH_4^+ on NO_3^- uptake is independent of its effects on root NRA.

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